ABSTRACT

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Title of rigorous thesis: Solubilisation, Purification and Reconstitution of Human 17beta-hydroxysteroid dehydrogenase type 3

Short-chain dehydrogenase/reductase superfamily is a large group of NADP(H)/NAD(H)-dependent oxidoreductases. Human SDR enzymes are classified into 47 families, including cytosolic and membrane-bound ones. One of them is a human membrane-bound enzyme 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), which participates in the biosynthesis of steroidal hormones and mainly catalyses the conversion of androstenedione to testosterone. The aims of this study were to find a suitable detergent for successful solubilisation, to purify the enzyme and to prepare a reconstitution system for further study of the 17β-HSD3 enzyme.

Microsomes containing overexpressed 17β-HSD3 were isolated from *Sf9* insect cells (*Spodoptera frugiperda)*. The first step was the solubilisation process, which involved detergent screening. Six detergents at different concentrations were tested: ASB 14-4, C12E8, DDM, CHAPS, Igepal CA-630 and Triton X-100. The detergent ASB 14-4 in 0.5% (w/v) concentration has been identified to be the most suitable one for the 17β-HSD3 solubilisation.

The next step was enzyme purification using the His_{10}-tag located on C-terminus of the 17β-HSD3 and Ni^{2+}-metal affinity chromatography (Ni^{2+}-IMAC). By this approach the purified enzyme 17β-HSD3 in the active form was obtained, which was then incorporated into custom prepared liposomes, whose phospholipid composition was based on the native membrane of the human liver endoplasmic reticulum.